Green Tea, Soy, and Mammographic Density in Singapore Chinese Women

Anna H. Wu,[1] Giske Ursin,[1] Woon-Puay Koh,[2] Renwei Wang,[3] Jian-Min Yuan,[3] Kei-Siong Khoo,[4] and Mimi C. Yu[3]

[1]University of Southern California, Los Angeles, California; [2]National University of Singapore, Singapore; [3]University of Minnesota, Minneapolis, Minnesota; and [4]National Cancer Centre, Singapore

Abstract

There is increasing evidence from observational studies that breast cancer risk is inversely associated with soy and green tea consumption. We investigated the effects of these two dietary agents on mammographic density, a well-established biomarker for breast cancer risk, in a cross-sectional analysis of mammograms and validated food frequency questionnaires from 3,315 Chinese women in Singapore. Percent mammographic density (PMD) was assessed using a reproducible computer-assisted method. We used generalized linear models to estimate PMD by intake of soy, green tea, and black tea while adjusting for potential confounders. Daily green tea drinkers showed statistically significantly lower PMD (19.5%) than non-tea drinkers (21.7%; *P* = 0.002) after adjusting for relevant covariates. This difference in PMD between daily green tea drinkers and non-tea drinkers remained statistically significant after adjustment for soy (*P* = 0.002); the effect was more apparent among lower soy consumers (Q1-Q3; 21.9% versus 19.4%; *P* = 0.002) than in higher (Q4) consumers (20.9% versus 19.5%; *P* = 0.32). Black tea intake was unrelated to PMD. Only among postmenopausal women who reported very high soy intake (Q4) compared with those with less soy intake was there any association noted between PMD and soy intake (18.9% versus 20.5%; *P* = 0.035). Following adjustment for green tea intake, the association between soy and PMD was no longer statistically significant (*P* = 0.52). Our findings suggest that both regular green tea and high soy intake may have beneficial effects on the breast; the effect of green tea on PMD may be stronger than the effect of soy. (Cancer Epidemiol Biomarkers Prev 2008;17(12):3358–65)

Introduction

There is accumulating evidence that breast cancer risk in Asians and Asian Americans is influenced by intake of soy (1, 2) and green tea (3). We recently conducted a meta-analysis of eight observational epidemiologic studies conducted in Asian populations with relatively complete assessment of dietary soy exposure and found a stepwise reduction in breast cancer risk with increasing soy intake. Compared with lowest soy intake (<5 mg/d isoflavones), the risk of breast cancer was reduced significantly by 12% in association with moderate intake (~10 mg/d isoflavones) and 29% in association with high intake (≥20 mg/d isoflavones; ref. 1). Although there are fewer studies on green tea and breast cancer risk, a combined meta-analysis of four studies showed a statistically significant, 22% reduction in risk between subjects in the highest versus non/lowest categories of green tea intake (3). A combined meta-analysis of 13 studies showed no significant relationship between breast cancer risk and black tea intake (3). Although most previous studies on breast cancer have primarily focused on the cancer prevention activity of soy or tea alone, we investigated the combined effects of soy and green tea on risk of breast cancer among Asian American women because both dietary compounds are flavonoids that are rich in antioxidants (4). Significantly, we reported earlier that the inverse association between green tea and breast cancer risk was clearest among women with low soy intake and the inverse association between soy and breast cancer was clearest among non-green tea drinker (4). We speculated that this pattern of joint effects is consistent with the notion of a shared pathway between green tea and soy intake in breast cancer development.

Mammographic density is a well-established breast cancer risk factor (5). Substantial evidence shows that mammographic density is influenced by markers of exposure to endogenous hormones and use of exogenous hormones. Specifically, percent density has been found consistently to decrease with increasing parity and with increasing body weight (5). Mammographic density also decreases when women undergo menopause (6) or are exposed to artificial reductions in circulating steroids or tamoxifen (7-9) and increases with use of replacement hormones, particularly the combined estrogen and progestin therapy (10). However, findings on the association between circulating hormone levels and mammographic density are not consistent (11-13). Few studies have examined the effect of soy on mammographic density; the strongest finding of an effect of soy on mammographic density was based on a pilot cross-sectional study of some 380 women we conducted among

Received 2/11/08; revised 8/18/08; accepted 9/19/08.

Grant support: National Cancer Institute grants R01CA102065, R01CA55069, R35CA53890, and R01CA80205.

Requests for reprints: Anna H. Wu, Department of Preventive Medicine, University of Southern California, 1441 Eastlake Avenue, Los Angeles, CA 90089. Phone: 323-865-0484; Fax: 323-865-0139. E-mail: annawu@usc.edu

doi:10.1158/1055-9965.EPI-08-0132

Chinese women in Singapore (14, 15). We are not aware of any data on mammographic density and intake of tea. We have conducted a large cross-sectional study among Singaporean Chinese to further investigate our previous finding on soy and mammographic density (14, 15) and to investigate the effects of green tea and black tea on mammographic density. Excluding the women who were part of the published pilot study (14, 15), the current study included all remaining, eligible women common to the following two population-based cohorts: the Singapore Chinese Health Study (SCHS; enrollment during 1993-1998) and the nationwide Singapore Breast Screening Project (SBSP; 1994-1997). We measured mammographic density using a highly reproducible computer-assisted method we have used extensively and have found to predict breast cancer risk (10, 15-17). We report below our findings on mammographic density and intake of soy, green tea, and black tea in this study population.

Subjects and Methods

This cross-sectional study was conducted among Chinese women in Singapore who were participants of two population-based cohort studies, the SCHS and the SBSP. The SCHS was a prospective investigation of the role of dietary factors in cancer etiology, which recruited 35,298 Chinese women and 27,959 men, ages 45 to 74 years during 1993 to 1998. Subjects were residents of government housing estates; during the enrollment period, 86% of the Singapore population resided in such housing facilities. The SBSP was a randomized clinical trial conducted among Singaporean women ages 50 to 69 years during 1994 to 1997. A total of 67,656 women were randomized to the mammography arm of the SBSP, and 28,231 (41.7%) women were screened. Participants in the SBSP were more likely to be married, to have a formal education, and to be employed outside the home (18). Education and parity were adjusted for in our analysis (see below). A computer linkage of the SBSP and SCHS databases identified 3,777 women common to both databases of whom the mammograms of 3,702 (98%) women were retrieved successfully. We have reported previously on a random subset (n = 380) of these women (14, 15); these women were excluded from the current study. The rationale for excluding these women was based on our intent to use the current study to either confirm or refute the association between soy intake and percent mammographic density (PMD) observed among women in the pilot study (14, 15). After excluding 6 women due to missing key variables (e.g., PMD or menopausal status) and 1 woman who was later found not to be a resident of Singapore, the remaining 3,315 women constituted the subjects of this cross-sectional analysis. Among our study subjects, the median time interval between dietary assessment conducted at entry to the SCHS and the mammogram screening was 1.2 years (interquartile range, 0.8-1.8), and most subjects gave their dietary histories before the mammogram screening.

At entry to the SCHS, a face-to-face interview was conducted in the subject's home by a trained interviewer using a structured questionnaire that asked about menstrual and reproductive history (women only), medical history, family history of cancer, and other lifestyle factors relevant to breast cancer. The questionnaire included a validated dietary food frequency questionnaire in which each subject was asked to estimate his/her usual intake frequencies and portion sizes for 165 food and beverage items in the past 12 months (19). Specific questions were asked about usual frequency of intake (never or hardly ever, 1-3 times a month, once a week, 2-3 times a week, 4-6 times a week, once a day, 2-3 times a day, 4-5 times a day, and ≥6 times a day) of seven nonfermented soy products, black tea, green tea, coffee, and other beverages. We expressed total soy intake in terms of grams of soy protein, soy isoflavones, and equivalent amounts of tofu per day (14, 15). We included only women without any history of cancer because lifestyle habits may change as a result of cancer diagnosis and the presence of tumor and/or cancer treatment may additionally influence mammographic density.

As part of the SBSP, participants completed a brief questionnaire that asked about demographic and body size characteristics, reproductive history, use of hormonal contraceptives, medical history, and use of various screening programs. For demographic data and other variables that were asked in both SBSP and SCHS, we found almost perfect agreement between the two studies. However, factors such as menopausal status and use of hormone therapy were likely to be strongly associated with mammographic density, so we used the demographic data collected at the same time the mammogram was done, as part of the SBSP, for these analyses.

All 3,315 women with available mammograms are included in our analysis of soy intake and mammographic density. However, in our analysis on green tea and black tea, we excluded 456 women who drank both types of tea regularly because we have found previously that black tea and green tea might have opposing effects on blood hormone levels (20). Specifically, estrone and estradiol levels were 19% and 10% higher, respectively, among exclusive regular black tea compared with nonregular tea drinkers, whereas the corresponding levels were 13% and 8% lower in regular green tea drinkers. Given the rather strong and opposing effects of green tea versus black tea drinking on circulating estrogens, established markers of breast cancer risk, we have chosen to exclude women consuming both types of tea from the tea/mammographic density analysis. Therefore, the final analysis on tea intake in relation to mammographic density was conducted on 2,859 women: 1,560 who did not drink any tea, 498 who drank only black tea, and 801 who drank only green tea.

Mammograms were assessed for density by Dr. Ursin using a highly reproducible computer-assisted method, which she has used extensively (10, 11, 15-17). In brief, subjects' mammograms were scanned using a Cobrascan 812T scanner (Radiographic Digital Imaging) and Adobe Photoshop software with the plug-in program Scanwizard 3.0.9. The outline of the breast was done by two research assistants trained by Dr. Ursin; their average readings were used. The readers outlined the entire breast using an outlining tool on the computer screen, and the computer counts the total number of pixels in the breast. This represents the total breast area, and this number was converted to cm^2. The density readings were done as follows: The reader draws a region of interest that excludes the pectoralis muscle and other artifacts and then uses a tinting tool to color the areas within the region of interest considered to contain mammographic densities. The software counts the number of tinted pixels within the region of interest, this represents the area with absolute density, and this number was converted to cm^2. All mammograms were organized into batches of 50, which included subjects of all age groups. Dr. Ursin read the mammograms by batches; the batch number was included as an adjustment covariate in all of our statistical analysis models (see below). The PMD is the dense area divided by the total breast area. Approximately 7% (n = 237) of the mammograms had duplicate readings and the reproducibility on percent density assessment was high (r = 0.97; P < 0.0001).

The institutional review boards at the University of Southern California, the University of Minnesota, the Singapore National Cancer Center, and the National University of Singapore had approved this study.

Statistical Methods. Dietary assessment was derived from baseline interviews of the SCHS. All other variables were derived from interviews conducted at the time of mammography. We used analysis of covariance methods to estimate least-square means (LSM) of PMD according to varying levels of intake of soy, green tea, and black tea with adjustment for multiple potential confounders including age at mammography (continuous; years), body mass index (BMI) at mammography (continuous; kg/m^2), education (no formal education, primary school, secondary school, A level/university), parity (0, 1-2, 3-4, ≥5), menopausal status at mammography (premenopausal, postmenopausal), use of postmenopausal hormone therapy (never, ex, current), total food energy (kcal), and batch number. Table 1 shows the LSM PMD in all study participants by age group, education, BMI, parity, and menopausal status. We show the LSM results in two ways in Table 2. In one method (method 1, Table 2), the LSM was calculated by weighing the two strata based on the number of premenopausal and postmenopausal women in each stratum, whereas, in the second method (method 2, Table 2), LSM was calculated by giving equal weights to the results in the subgroups of premenopausal and postmenopausal women, respectively. The latter method was used in our previous pilot study (15). As shown in Table 2, the actual values of PMD differ between the two methods, but the relative differences in PMD between the exposure levels of interest are similar across the two methods. Results based on method 1 are reported in the other tables (Tables 1, 3, and 4). Trend tests were conducted using actual values of the relevant interval-scale variables. In our previous study, we noted that the clearest difference in PMD was between the highest (Q4) soy intake group and the other quartiles (Q1-Q3) combined (15). Therefore, we are prompted by our previous finding to compare results of Q1 to Q3 combined versus Q4 in this analysis. We present the results on soy, green tea, and black tea intake for all women and separately for premenopausal and postmenopausal women. Analysis of covariance methods were used to test the simultaneous effects of green tea and soy intake on PMD while adjusting for the effects of age, education, BMI, parity, and menopausal status. We considered P values < 0.05 to be statistically significant. All analyses were done using the SAS software package version 9.13 (SAS Institute).

Results

Table 1 shows the LSM PMD in all study participants (n = 3315) included in the soy analysis by age group, menopausal status, education, BMI, and parity; these variables were mutually adjusted for each other. Results were essentially identical when we repeated the same analysis among women included in the cross-sectional analysis by tea intake (n = 2,859; excluding the 456 women who drank both green and black tea; data not shown). We confirm the significant trends of decreasing density with increasing age, being postmenopausal, higher educational attainment, increasing body weight, and increasing parity, which we (15) and many others (5) have reported. Mean intake of soy isoflavone was unrelated to age, BMI, parity, and menopausal status, but intake was higher among subjects with more education. Prevalence of daily green tea drinker was lower among women who were postmenopausal, had ≥5 births, and were heavier (above median BMI) but was unrelated to age and associated with education in a U-shaped manner (lower in women with no education or more education; Table 1); these variables were adjusted for in all subsequent analyses.

Table 2 shows the relationship between mean PMD and soy intake in all subjects and separately in premenopausal and postmenopausal women after adjustment for potential confounders including age, parity, body size, and other covariates. Women with lower (Q1-Q3, <23.5 mg/d isoflavones) soy intake displayed similar PMD, but those levels were higher than that associated with the highest quartile of soy intake (>23.5 mg/d isoflavones; P = 0.055). This difference in PMD by soy intake was confined to postmenopausal women; PMD was 18.9% for postmenopausal women in the highest quartile of soy intake and 20.5% for those in the lower three quartiles of soy intake (P = 0.035; $P_{\text{interaction}}$ = 0.30).

Table 3 shows the PMD by intake of green tea and black tea after adjustment for potential confounders. In all subjects, mean percent density was higher in non-tea drinkers (21.7%) than in green tea drinkers [19.5%; 95% confidence interval (95% CI), 18.3-20.6; P = 0.002]. This pattern of lower percent density in association with daily green tea intake was observed in both premenopausal and postmenopausal women, although, due to the lower number of premenopausal women (n = 215), the results were statistically significant only in postmenopausal women (P_{trend} = 0.003; $P_{\text{interaction}}$ = 0.83). The inverse association between green tea drinking and percent density remained statistically significant when we included only postmenopausal women who were never users of hormone therapy (19.3% for non-tea drinkers and 17.8% in green tea drinkers; P = 0.045; data not shown). In contrast, mammographic density was unrelated to black tea intake in all subjects or separately in premenopausal or postmenopausal women (Table 3).

We evaluated the effects of soy and green tea on mammographic density simultaneously (Table 4). In analyses of all subjects, PMD was 2.2% lower in green

Table 1. PMD, mean soy intake, and prevalence of daily green tea drinkers in study participants by selected characteristics

	All subjects		Mean soy isoflavones* (mg/d)	% Daily green tea drinker †
	n = 3,315	Mean (95% CI) PMD ‡ (%)		
Age (y)				
50	69	31.8 (27.9-35.7)	19.3	11.6
51-55	1,180	24.7 (23.7-23.6)	18.7	11.6
56-60	1,160	20.4 (19.5-21.4)	18.1	9.9
61-65	806	17.6 (16.5-18.8)	17.5	10.4
≥66	100	15.6 (12.4-18.9)	18.2	8.0
P_{trend}		0.0001	0.15	0.617
Education				
No formal education	1,404	20.5 (19.6-21.4)	17.5	8.1
Primary school	1,355	21.8 (20.9-22.6)	18.3	12.6
Secondary school	454	22.3 (20.7-23.9)	19.3	12.6
A level/university	102	23.4 (20.2-26.6)	21.0	9.8
P_{trend}		0.012	0.005	<0.001
BMI (kg/m^2)				
<20	379	31.9 (30.3-33.5)	18.6	5.0
20 to <24	1,227	23.3 (22.4-24.2)	17.8	9.1
24 to <28	1,174	18.7 (17.8-19.6)	18.2	13.0
>28	535	15.3 (13.9-16.7)	18.7	12.7
P_{trend}		0.0001	0.54	<0.001
Parity				
0	213	34.0 (31.8-36.3)	17.3	11.7
1-2	737	27.3 (26.0-28.5)	17.8	11.3
3-4	1,385	20.3 (19.5-21.2)	17.9	11.8
≥5	980	15.6 (14.5-16.7)	19.0	8.3
P_{trend}		0.0001	0.09	0.041
Menopausal status				
Premenopausal	320	28.0 (26.1-30.0)	17.0	13.8
Postmenopausal	2,995	20.6 (20.0-21.2)	18.3	10.3
P_{trend}		0.0001	0.15	0.056

*Adjusted for age at mammography (if applicable), BMI at mammography (kg/m^2; if applicable), education (no formal education, primary school, secondary school, A level/university; if applicable), parity (0,1-2, 3-4, ≥5; if applicable), and menopausal status (pre, post; if applicable), and total calories.
†From χ^2 test. Of 3,315 subjects, 352 were daily green tea drinkers.
‡Adjusted for age at mammography (if applicable), BMI at mammography (kg/m^2; if applicable), education (no formal education, primary school, secondary school, A level/university; if applicable), parity (0, 1-2, 3-4, ≥5; if applicable), and menopausal status (pre, post; if applicable).

tea drinkers than in non-green tea drinkers after adjusting for soy intake and other covariates (P = 0.002), whereas PMD did not differ significantly between high (Q4) versus lower (Q1-Q3) soy consumers (P = 0.52) after adjusting for green tea intake and other covariates. Among non-green tea drinkers, there was a full percentage point difference in PMD between those with low soy (Q1-Q3; mean, 21.9%) and those who had higher (Q4) soy intake (mean, 20.9%; Table 4). These patterns of results were similar in postmenopausal women (Table 4, bottom).

Discussion

Intake of soy and green tea has emerged as two dietary agents that may have contributed, in part, to the traditionally lower risk of breast cancer in Asian populations (1-3). To our knowledge, this is the first population-based study that has investigated simultaneously the association between mammographic density and intake of soy and tea. Mammographic density is considered by some to be the best surrogate endpoint for breast cancer risk (5). An association between high mammographic density and breast cancer risk has been shown consistently in Whites and non-Whites including Asians (16).

In this cross-sectional study with over 3,300 Chinese women from the SCHS, we found a small effect of soy intake on PMD (Table 2); this finding is compatible with our pilot study results (14, 15). Among postmenopausal women in our pilot study, the difference in mean PMD between women in the Q1 to Q3 (23.6%) versus Q4 (21.3%) groups was 2.3% (15). The corresponding figures from the current study is a difference of 1.5%; 24.0% in Q1 to Q3 versus 22.5% in Q4 (Table 2, column 2 of PMD). The methods we used to determine PMD in our pilot study and this current study are identical. Reasons for a weaker effect in the current study are not apparent, but the accumulating data suggest that the effect of soy on breast cancer risk tends to be stronger than the effects of soy on mammographic density and endogenous hormone levels, both of which are established intermediate markers of breast cancer risk (1). The generally weaker effects and the lack of clear dose-response relationship between soy and PMD suggest that the beneficial effects of soy on breast cancer risk are not confined to mechanisms that also affect breast density. Nonhormonal mechanisms (e.g., antiproliferative and anti-inflammatory) should be investigated in future studies.

The timing, type, and amounts of soy intake have emerged to be important codeterminants of breast cancer risk (1). The quartile cut points of soy intake in this Singapore population are compatible with moderate and high soy intake levels reported in our meta-analysis of soy and breast cancer risk (1). However, usual adult soy intake was unrelated to mammographic density in other cross-sectional studies conducted in Hawaii (21, 22) and Japan (23). In one study conducted in Hawaii, high soy intake (34.8 g soy foods or ~10 mg/d isoflavones) was associated with lower mammographic density among Chinese and Japanese women but was associated with higher mammographic density among Caucasians and Hawaiians, although none of these findings were statistically significant (21). In another study conducted in Hawaii, soy intake during childhood and adulthood had differing effects on breast density (22). Although the amount of soy intake was not presented in the second Hawaiian study, women with high soy intake during adult life but low intake during childhood exhibited significantly higher densities, whereas women with high soy intake during early life but low intake in adulthood showed lowest densities (22). These latter results suggest that timing of the soy exposure may play a role in soy's eventual effect on a soy-consuming woman's mammographic density. In the Japanese study (23), breast density did not differ significantly between women in the lowest versus highest quartiles of soy intake. However, soy intake was uniformly high in this Japanese study; the average daily soy intake was ≤28.6 mg isoflavones in the lowest quartile versus >56.7 mg isoflavones in the highest quartile of intake (23). Controlled intervention studies ranging from 1 to 2 years conducted in mostly premenopausal or perimenopausal women have found no significant change in PMD among women randomized to soy protein or red clover-derived soy supplements compared with those treated with a placebo (24-26). If type of soy (tofu versus soy as fillers/extenders in processed foods) and/or early-life soy intake are indeed important codeterminants of the beneficial effects of soy on breast health, they may explain, in part, the generally null findings in short-term intervention studies.

No consistent effects of soy on circulating hormone levels have been reported in short-term soy intervention studies conducted in premenopausal or postmenopausal women (see review in refs. 27, 28). However, there is suggestion from some cross-sectional studies, including one we conducted among Singapore Chinese women, of a significant reduction in estrone levels in association with the highest level of soy intake (29). In a cross-sectional study conducted in the Multiethnic Cohort of Hawaii and Los Angeles County, high soy intake was associated with reduced levels of estradiol, estrone, and testosterone; the finding in relation to testosterone was statistically significant (30). It has been suggested that differences in a woman's ability to produce equol may influence the extent to which her soy consumption can affect her risk of breast cancer development (1, 31). At present, it is unclear whether failure to take account of the differences in soy metabolism between individuals has contributed to the inconsistencies between studies on intermediate markers of breast cancer risk, including mammographic density.

Green tea drinking significantly influences mammographic density; there was an absolute difference of ~2% in mammographic density between daily green tea drinkers and nondrinkers (Table 3). In all subjects

Table 2. Adjusted PMD and 95% CI according to levels of dietary soy isoflavones

Soy isoflavones (mg/d)	n (total = 3,315)	Mean (95% CI) PMD (%)	
		Method 1*	Method 2 †
All subjects			
Q1 (<7.7)	829	21.0 (19.9-22.2)	26.3 (24.8-27.8)
Q2 (>7.7-14.1)	829	22.4 (21.3-23.6)	27.7 (26.2-29.2)
Q3 (>14.1 to ≤23.5)	829	21.5 (20.3-22.6)	26.7 (25.2-28.2)
Q4 (>23.5)	828	20.4 (19.2-21.6)	25.7 (21.1-27.2)
P_{trend}		0.280	0.280
Q1 + Q2 + Q3	2,487	21.7 (21.0-22.3)	26.9 (25.8-28.1)
Q4	828	20.4 (19.2-21.6)	25.7 (21.1-27.2)
P_{trend}		0.055	0.055
Premenopausal			
Q1 (<7.7)	70	32.2 (27.7-36.7)	35.1 (30.2-40.4)
Q2 (>7.7-14.1)	87	32.0 (28.1-35.9)	34.9 (30.3-39.5)
Q3 (>14.1 to ≤23.5)	77	33.4 (29.3-37.5)	36.3 (31.3-41.3)
Q4 (>23.5)	86	33.8 (29.6-38.0)	36.7 (31.9-41.5)
P_{trend}		0.565	0.565
Q1 + Q2 + Q3	234	32.5 (30.1-34.9)	35.4 (32.0-38.8)
Q4	86	33.7 (29.6-37.9)	36.6 (31.9-41.3)
P_{trend}		0.583	0.583
Postmenopausal			
Q1 (<7.7)	759	19.8 (18.6-21.0)	23.3 (21.8-24.9)
Q2 (>7.7-14.1)	742	21.4 (20.3-22.6)	24.9 (23.5-26.4)
Q3 (>14.1 to ≤23.5)	752	20.2 (19.0-21.4)	23.7 (22.2-25.2)
Q4 (>23.5)	742	19.0 (17.7-20.2)	22.4 (21.0-24.0)
P_{trend}		0.218	0.218
Q1 + Q2 + Q3	2,253	20.5 (19.8-21.2)	24.0 (22.9-25.2)
Q4	742	18.9 (17.7-20.2)	22.5 (21.0-24.0)
P_{trend}		0.035	0.035

NOTE: Adjusted for age at mammography, BMI at mammography (kg/m^2), education (no formal education, primary school, secondary school, A level/university), menopausal status (for all subjects), parity (0,1-2, 3-4, ≥5), and total calories (kcal).

*The LSM was calculated using an analysis of covariance with weighted average method.

†The LSM was calculated using an analysis of covariance by assigning equal weights to the strata included in the analysis. This method was used in our previous study (15).

Table 3. Adjusted PMD and 95% CI according to intake levels of green tea and black tea separately

	Green tea		Black tea	
	n (total = 2,361)*	Mean (95% CI) PMD (%)	n (total = 2,058)†	Mean (95% CI) PMD (%)
All subjects				
Non-tea drinkers	1,560	21.7 (20.9-22.5)	1,560	21.9 (21.1-22.7)
Less than daily	554	19.2 (17.9-20.6)	356	21.0 (19.3-22.7)
Daily	247	20.0 (18.0-22.1)	142	21.0 (18.3-23.8)
P_{trend}		0.008		0.358
Non-tea drinkers	1,560	21.7 (20.9-22.5)	1,560	21.9 (21.1-22.7)
Drinkers	801	19.5 (18.3-20.6)	498	21.0 (19.6-22.5)
P_{trend}		0.002		0.316
Premenopausal				
Non-tea drinkers	138	33.4 (30.5-36.4)	138	33.0 (29.9-36.0)
Less than daily	52	30.5 (25.6-35.4)	28	34.0 (27.1-41.0)
Daily	25	29.8 (22.8-36.7)	17	32.8 (23.9-41.7)
P_{trend}		0.282		0.914
Non-tea drinkers	138	33.4 (30.5-36.4)	138	33.0 (30.0-36.0)
Drinkers	77	30.3 (26.3-34.2)	45	33.6 (28.1-39.0)
P_{trend}		0.328		0.861
Postmenopausal				
Non-tea drinkers	1,422	20.6 (19.7-21.4)	1,422	20.8 (20.0-21.7)
Less than daily	502	18.0 (16.6-19.4)	328	19.8 (18.0-21.5)
Daily	222	19.1 (17.0-21.2)	125	20.0 (17.1-22.9)
P_{trend}		0.017		0.347
Non-tea drinkers	1,422	20.6 (19.7-21.4)	1,422	20.8 (20.0-21.7)
Drinkers	724	18.4 (17.2-19.5)	453	19.8 (18.3-21.3)
P_{trend}		0.003		0.289

NOTE: Adjusted for age at mammography, BMI at mammography (kg/m^2), education (no formal education, primary school, secondary school, A level/university), menopausal status (pre, post in analysis for all subjects), parity (0,1-2, 3-4, ≥5), and total calories (kcal).
*954 subjects who drank black tea were excluded from this analysis.
†1,257 subjects who drank green tea were excluded form this analysis.

combined, the effect of green tea on mammographic density was only apparent among women with lower soy intake (Table 4). Interestingly, this possible modifying effect of soy on the green tea/PMD association lends support to an earlier observation we noted among Asian American women of Los Angeles County. In our Los Angeles Asian Breast Cancer Study, we noted a stronger effect of green tea on breast cancer risk among low versus high consumers of dietary soy (4). We reasoned that these empirical findings suggest the presence of common mechanistic pathway(s) between soy and green tea exposure, with a threshold dose, in relation to breast cancer development. The effect of green tea on mammographic density seems to be more modest than that (4-6%) associated with use of menopausal hormones containing estrogen and progestin (10).

Table 4. Adjusted PMD and 95% CI according to combined levels of green tea intake and dietary soy isoflavones in all subjects and postmenopausal women

Soy isoflavones (mg/d)	Non-green tea drinker		Green tea drinker		Total*
	n	Mean (95% CI) PMD	n	Mean (95% CI) PMD	
		All subjects†			
Q1 + Q2 + Q3	1,249	21.9 (21.0-22.8)	583	19.4 (18.1-20.7)	21.1 (20.3-22.5)
Q4	311	20.9 (19.1-22.8)	218	19.5 (17.3-21.7)	20.5 (19.0-20.6)
Total‡		21.7 (20.9-22.5)		19.5 (18.3-20.6)	
		Postmenopausal women§			
Q1 + Q2 + Q3	1,130	20.7 (19.7-21.6)	531	18.3 (16.9-19.7)	19.9 (19.1-20.7)
Q4	292	20.2 (18.2-22.1)	193	18.4 (16.1-20.8)	19.6 (18.1-21.1)
Total‡		20.5 (19.7-21.4)		18.4 (17.2-19.5)	

NOTE: Adjusted for age at mammography, BMI at mammography (kg/m^2), education(no formal education, primary school, secondary school, A level/university), parity (0,1-2, 3-4, ≥5), total calories (kcal), and menopausal status (pre, post, in analysis for all subjects).
*Tea-adjusted PMD (95% CI) for low (Q1 + Q2 + Q3) versus high (Q4) soy intake. P (low soy versus high soy) = 0.52 for all subjects and 0.76 for postmenopausal women only.
† This analysis included 2,361 subjects after excluding 954 subjects who drank black tea.
‡ Soy-adjusted PMD (95% CI) for non-green tea drinker and green tea drinker. P (non-green tea versus green tea) = 0.002 for all subjects and 0.003 for postmenopausal women only. Dietary soy did not modify the green tea MD association ($P_{interaction}$ = 0.30, all subjects).
§This analysis included 2,171 postmenopausal women after excluding 849 who drank black tea. Analyses of joint effects of soy and tea were not examined separately in premenopausal women because of the relatively small numbers in some of the cells.

It is noteworthy that the effect of tea on mammographic density was specific to green tea as black tea intake was unrelated to mammographic density. These findings of a specific inverse association between green tea intake and mammographic density mirror the epidemiologic findings on tea intake and breast cancer (3) and the limited data on circulating hormones and tea intake (20). In a meta-analysis of 13 case-control and cohort studies, risk of breast cancer was unrelated to intake of black tea but was inversely associated with intake of green tea (3). This inverse association with green tea persists with information from two additional studies (32, 33). When we update a meta-analysis of these 6 studies (4 cohort and 2 case-control studies), high green tea intake is associated with a 27% (summary odds ratio, 0.73; 95% CI, 0.53-0.86) reduction in risk of breast cancer.

The biological basis of mammographic density and the mechanism by which high mammographic density is associated with elevated breast cancer risk is not well understood. Mammographic density may reflect cumulative exposure to estrogens (5) and there is some evidence that green tea catechin may alter estradiol action by disrupting estradiol biosynthesis and/or metabolism (34). High mammographic density may reflect an increased rate of cellular proliferation due to the increased number of epithelial cells (35). Interestingly, in a spontaneous tumor model [C3(1)/SV40 mice model], green tea administration (0.5% Polyphenon E) inhibited the proliferation of mammary ductal epithelial cells, suppressed the invasiveness of ductal lesions, and disrupted angiogenesis in ductal epithelial tumor and surrounding stromal cells (36). These findings of antiproliferative and antiangiogenic effects of green tea on both ductal and stromal epithelial cells may be particularly relevant to our findings on mammographic density. Previously, we reported a stronger inverse green tea-breast cancer association among women possessing the high-activity angiotensin-converting enzyme genotype than women with the low-activity angiotensin-converting enzyme genotype (32), suggesting that green tea catechin may exert their chemopreventive effect through an angiotensin II-driven pathway. Lowering of angiotensin II levels by angiotensin-converting enzyme inhibitors has been shown to suppress vascular endothelial growth factor-induced angiogenesis and inhibit tumor growth *in vivo* (37, 38). Thus, the antiangiogenic effects of green tea in the rodent studies conducted by Leong et al. (36) provide compelling supportive data that one of the mechanisms by which green tea catechins may modulate breast cancer risk is via this antiangiogenic pathway.

Some methodologic strengths and limitations of this investigation should be considered. A strength of this study is the large sample size and that the evaluation of mammographic breast densities was conducted using a highly reproducible computer-assisted method (16, 17). Mammograms were reviewed without any knowledge of demographic or lifestyle factors (including tea drinking habits) of the study participants; thus, any misclassification of PMD would be presumed to be random and to bias the results toward the null. The distribution of breast density in this population is also comparable with that reported in other studies. We found an average 16% difference in percent density among women ages ≤50 years compared with older women (ages ≥66 years) in our study (breast density was 31.8% versus 15.6%, respectively). In a study of mostly Whites and Japanese women in Hawaii, there was a 12% difference in percent density between women ages 50 years and those ages ≥66 years (22). In addition, information on tea and soy intake was obtained largely before mammographic screening. However, we only asked about usual frequency of tea drinking habits but did not ask about duration of tea drinking habits or other variables of tea drinking including brewing conditions (tea strength) and temperature of the tea beverage, all of which may affect the final level of tea catechins in the brew. Thus, limitations in our assessment and the possibility of random misclassification may have contributed to the small differences between daily green tea drinkers and nondaily green tea drinkers (random misclassification should only bias the results toward the null, so that any effect on mammographic density by green tea intake may actually be stronger).

To our knowledge, this is the first study to examine the separate and combined effects of green tea and soy on mammographic density. We observed inverse associations between intake of green tea and soy and mammographic density among Chinese women in Singapore. The effect associated with soy was found only in postmenopausal women and only among high soy consumers with little evidence of a clear dose-response relationship. It is important that one puts into proper perspective the reduction of ∼2% in PMD between drinkers and nondrinkers of green tea and high versus low consumers of dietary soy. Change of ∼4% to 6% in PMD has been reported with use of exogenous hormones (7, 10). Specifically, tamoxifen use has been associated with ∼4% to 6% mammographic density reduction (7), whereas use of menopausal hormones containing estrogen and progestin has been associated with a comparable magnitude of increase in mammographic density (10). Given the magnitude of differences measured with these drug regimens, the findings of our study suggest that regular green tea intake and high soy intake may have clinically relevant beneficial effects on the breast.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest. Reprints will not be available.

Acknowledgments

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Siew-Hong Low for supervising the fieldwork of the SCHS; Kazuko Arakawa for the development of the cohort study database; Matthew Growdon, Pamela Chia, and Myra Chai for digitizing the ≥3,300 mammogram files; and Matthew Growdon for outlining the boundaries of these mammograms.

References

1. Wu AH, Yu MC, Tseng CC, Pike MC. Epidemiology of soy exposures and breast cancer risk. Br J Cancer 2008;98:9–14.
2. Trock BJ, Hilakivi-Clarke L, Clarke R. Meta-analysis of soy intake and breast cancer risk. J Natl Cancer Inst 2006;98:459–71.

3. Sun CL, Yuan JM, Koh WP, Yu MC. Green tea, black tea and breast cancer risk: a meta-analysis of epidemiological studies. Carcinogenesis 2006;27:1310–5.
4. Wu AH, Yu MC, Tseng CC, Hankin J, Pike MC. Green tea and risk of breast cancer in Asian Americans. Int J Cancer 2003;106:574–9.
5. Boyd NF, Lockwood GA, Byng JW, Tritchler DL, Yaffe MJ. Mammographic densities and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1998;7:1133–44.
6. Boyd N, Martin L, Stone J, et al. A longitudinal study of the effects of menopause on mammographic features. Cancer Epidemiol Biomarkers Prev 2002;11:1048–53.
7. Cuzick J, Warwick J, Pinney E, Warren RM, Duffy SW. Tamoxifen and breast density in women at increased risk of breast cancer. J Natl Cancer Inst 2004;96:621–8.
8. Spicer DV, Ursin G, Parisky YR, et al. Changes in mammographic densities induced by a hormonal contraceptive designed to reduce breast cancer risk. J Natl Cancer Inst 1994;86:431–6.
9. Brisson J, Brisson B, Cote G, et al. Tamoxifen and mammographic breast densities. Cancer Epidemiol Biomarkers Prev 2000;9:911–5.
10. Greendale GA, Reboussin BA, Slone S, et al. Postmenopausal hormone therapy and change in mammographic density. J Natl Cancer Inst 2003;95:30–7.
11. Greendale GA, Palla SL, Ursin G, et al. The association of endogenous sex steroids and sex steroid binding proteins with mammographic density: results from the Postmenopausal Estrogen/Progestin Interventions Mammographic Density Study. Am J Epidemiol 2005;162:826–34.
12. Bremnes Y, Ursin G, Bjurstam N, et al. Endogenous sex hormones, prolactin and mammographic density in postmenopausal Norwegian women. Int J Cancer 2007;121:2506–11.
13. Warren R, Skinner J, Sala E, et al. Associations among mammographic density, circulating sex hormones, and polymorphisms in sex hormone metabolism genes in postmenopausal women. Cancer Epidemiol Biomarkers Prev 2006;15:1502–8.
14. Jakes RW, Duffy SW, Ng FC, et al. Mammographic parenchymal patterns and self-reported soy intake in Singapore Chinese women. Cancer Epidemiol Biomarkers Prev 2002;11:608–13.
15. Ursin G, Sun CL, Koh WP, et al. Associations between soy, diet, reproductive factors, and mammographic density in Singapore Chinese women. Nutr Cancer 2006;56:128–35.
16. Ursin G, Ma H, Wu AH, et al. Mammographic density and breast cancer in three ethnic groups. Cancer Epidemiol Biomarkers Prev 2003;12:332–8.
17. Ursin G, Astrahan MA, Salane M, et al. The detection of changes in mammographic densities. Cancer Epidemiol Biomarkers Prev 1998;7:43–7.
18. Ng EH, Ng FC, Tan PH, et al. Results of intermediate measures from a population-based, randomized trial of mammographic screening prevalence and detection of breast carcinoma among Asian women: the Singapore Breast Screening Project. Cancer 1998;82:1521–8.
19. Hankin JH, Stram DO, Arakawa K, et al. Singapore Chinese Health Study: development, validation, and calibration of the quantitative food frequency questionnaire. Nutr Cancer 2001;39:187–95.
20. Wu AH, Arakawa K, Stanczyk FZ, et al. Tea and circulating estrogen levels in postmenopausal Chinese women in Singapore. Carcinogenesis 2005;26:976–80.
21. Maskarinec G, Meng L. An investigation of soy intake and mammographic characteristics in Hawaii. Breast Cancer Res 2001;3:134–41.
22. Maskarinec G, Pagano I, Lurie G, Kolonel LN. A longitudinal investigation of mammographic density: the multiethnic cohort. Cancer Epidemiol Biomarkers Prev 2006;15:732–9.
23. Nagata C, Matsubara T, Fujita H, et al. Associations of mammographic density with dietary factors in Japanese women. Cancer Epidemiol Biomarkers Prev 2005;14:2877–80.
24. Atkinson C, Oosthuizen W, Scollen S, et al. Modest protective effects of isoflavones from a red clover-derived dietary supplement on cardiovascular disease risk factors in perimenopausal women, and evidence of an interaction with ApoE genotype in 49-65 year-old women. J Nutr 2004;134:1759–64.
25. Maskarinec G, Takata Y, Franke AA, Williams AE, Murphy SP. A 2-year soy intervention in premenopausal women does not change mammographic densities. J Nutr 2004;134:3089–94.
26. Maskarinec G, Williams AE, Carlin L. Mammographic densities in a one-year isoflavone intervention. Eur J Cancer Prev 2003;12:165–9.
27. Maskarinec G, Franke AA, Williams AE, et al. Effects of a 2-year randomized soy intervention on sex hormone levels in premenopausal women. Cancer Epidemiol Biomarkers Prev 2004;13:1736–44.
28. Wu AH, Stanczyk FZ, Martinez C, et al. A controlled 2-mo dietary fat reduction and soy food supplementation study in postmenopausal women. Am J Clin Nutr 2005;81:1133–41.
29. Wu AH, Stanczyk FZ, Seow A, Lee HP, Yu MC. Soy intake and other lifestyle determinants of serum estrogen levels among postmenopausal Chinese women in Singapore. Cancer Epidemiol Biomarkers Prev 2002;11:844–51.
30. Setiawan VW, Haiman CA, Stanczyk FZ, Le Marchand L, Henderson BE. Racial/ethnic differences in postmenopausal endogenous hormones: the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev 2006;15:1849–55.
31. Fuhrman BJ, Teter BE, Barba M, et al. Equol status modifies the association of soy intake and mammographic density in a sample of postmenopausal women. Cancer Epidemiol Biomarkers Prev 2008;17:33–42.
32. Yuan JM, Koh WP, Sun CL, Lee HP, Yu MC. Green tea intake, ACE gene polymorphism and breast cancer risk among Chinese women in Singapore. Carcinogenesis 2005;26:1389–94.
33. Zhang M, Holman CD, Huang JP, Xie X. Green tea and the prevention of breast cancer: a case-control study in Southeast China. Carcinogenesis 2007;28:1074–8.
34. Stuart EC, Scandlyn MJ, Rosengren RJ. Role of epigallocatechin gallate (EGCG) in the treatment of breast and prostate cancer. Life Sci 2006;79:2329–36.
35. Hawes D, Downey S, Pearce CL, et al. Dense breast stromal tissue shows greatly increased concentration of breast epithelium but no increase in its proliferative activity. Breast Cancer Res 2006;8:R24.
36. Leong H, Mathur PS, Greene GL. Inhibition of mammary tumorigenesis in the C3(1)/SV40 mouse model by green tea. Breast Cancer Res Treat 2008;107:359–69.
37. Yasumatsu R, Nakashima T, Masuda M, et al. Effects of the angiotensin-I converting enzyme inhibitor perindopril on tumor growth and angiogenesis in head and neck squamous cell carcinoma cells. J Cancer Res Clin Oncol 2004;130:567–73.
38. Yoshiji H, Kuriyama S, Kawata M, et al. The angiotensin-I-converting enzyme inhibitor perindopril suppresses tumor growth and angiogenesis: possible role of the vascular endothelial growth factor. Clin Cancer Res 2001;7:1073–8.